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PROVISIONAL APPLICATION COVER SHEET

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Docket Number	1435.033PRV	Type a plus sign (+) inside this box >	+
Customer No.	21186	Confirmation No.	22154 U.S. PTO 60/540867

INVENTOR(s)/APPLICANT(s)					
Name (last, first, middle initial)			RESIDENCE (CITY, AND EITHER STATE OR FOREIGN COUNTRY)		
Uhrich, Kathryn E.			Plainfield, NJ		
TITLE OF THE INVENTION (280 characters max)					
FOLIC ACID-CONJUGATED AMPHIPHILIC STAR-LIKE MACROMOLECULES (FA-ASMs) AS A TARGETED ANTI-CANCER DRUG DELIVERY SYSTEM					
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STATE	Minnesota	ZIP CODE	55402	COUNTRY	United States of America
ENCLOSED APPLICATION PARTS (check all that apply)					
XXX	Specification	Number of Pages	8	XXX	Small Entity Status Claimed
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XXX	The Commissioner is hereby authorized to charge the provisional application filing fee and any additional required fees or credit overpayment to Deposit Account Number: <u>19-0743</u>				

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE

Monique M. Perdok

Date January 30, 2004

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REGISTRATION NO. 42,989

PROVISIONAL APPLICATION FILING ONLY

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In **PROVISIONAL** Patent Application of: Kathryn E. Uhrich

Title: **FOLIC ACID-CONJUGATED AMPHIPHILIC STAR-LIKE MACROMOLECULES (FA-ASMs) AS A TARGETED ANTI-CANCER DRUG DELIVERY SYSTEM**

Docket No.: 1435.033PRV

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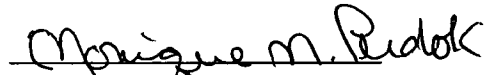
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(NEW FILING)

Folic Acid-Conjugated Amphiphilic Star-Like Macromolecules (FA-ASMs) as a Targeted Anti-Cancer Drug Delivery System

Background of the Invention

Currently, there is a in the art need for novel anti-cancer compounds/compositions and therapies.

Summary of the Invention

The present invention provides a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound.

The invention also provides a pharmaceutical composition comprising folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound and a pharmaceutically acceptable diluent or carrier.

The invention also provides a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound or a pharmaceutically acceptable salt thereof for use in medical therapy.

A method to target an anti-cancer compound to a cancer cell comprising, contacting said cell with a folic acid-conjugated amphiphilic star-like macromolecule encapsulating said anti-cancer compound.

A method to treat cancer comprising administering to a mammal a therapeutically effective amount of a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound.

The invention also provides the use of a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound or a pharmaceutically acceptable salt thereof to prepare a medicament useful for targeting an anti-cancer compound to a cancer cell.

The invention also provides the use of a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound or a pharmaceutically acceptable salt thereof to prepare a medicament useful for treating cancer.

The invention also provides synthetic intermediates and procedures described

herein that are useful for preparing a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound or a pharmaceutically acceptable salt thereof.

Processes and intermediates useful for preparing a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound or for preparing intermediates useful for preparing a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound are provided as further embodiments of the invention

Detailed Description

As used herein, "a method to treat cancer" includes treating, preventing or inhibiting cancer and/or the symptoms of cancer.

Amphiphilic star-like macromolecules can be prepared in a manner similar to that disclosed in WO 03/005959 (PCT/US02/21923), which is incorporated herein by reference.

In cases where compounds are sufficiently basic or acidic to form acid or base salts, use of the compounds as salts may be appropriate. Examples of acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The ability of a compound of the invention to treat cancer and/or target anti-cancer compounds to cancer cells may be determined using pharmacological models which are well known to the art, or using the assays described herein below.

Anti-cancer compounds (e.g., carcinomas, sarcomas, leukemias and cancers derived from cells of the nervous system), including anti-neoplastic compounds, useful in the present invention include, but are not limited to, 6-azauridine, 6-diazo-5-oxo-L-norleucine, 6-mercaptopurine, aclacinomycin(s), ancitabine, anthramycin, azacitadine, azaserine, bleomycin(s),

capecitabine, carubicin, carzinophillin A, chlorozotocin, chromomycin(s), cladribine, cytarabine, daunorubicin, denopterin, docetaxel, doxifluridine, doxorubicin, edatrexate, eflornithine, elliptinium, enocitabine, epirubicin, etoposide, floxuridine, fludarabine, gemcitabine, idarubicin, mannomustine, melphalan, menogaril, methotrexate, mitobronitol, mitolactol, mitomycin C, mitoxantrone, mopidamol, mycophenolic acid, nogalamycin, olivomycin(s), paclitaxel, pentostatin, peplomycin, pirarubicin, piritrexim, plicamycin, podophyllinic acid 2-ethylhydrazine, prednimustine, procarbazine, pteropterin, puromycin, ranimustine, streptonigrin, streptozocin, teniposide, thiamiprine, thioguanine, Tomudex® (N-[[5-[[[(1,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]methylamino]-2-thienyl]carbonyl]-L-glutamic acid), toptecan, trimetrexate, tubercidin, ubenimex, vinblastine, vindesine, vinorelbine, zorubicin and the like.

The entire disclosure of the following manuscript entitled “Folic acid-conjugated amphiphilic star-like macromolecules (FA-ASMs) as a targeted anti-cancer drug delivery system” is included as part of this provisional patent application.

Folic acid-conjugated amphiphilic star-like macromolecules (FA-ASMs) as a targeted anti-cancer drug delivery system

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ABSTRACT SUMMARY

Folic acid-conjugated amphiphilic star-like macromolecules (FA-ASMs) were synthesized and evaluated in terms of their drug encapsulation ability and cytotoxicity. Drug loading studies showed that doxorubicin can be successfully incorporated into the micelles, whereas cytotoxicity studies revealed that FA-ASMs didn't show significant cytotoxicity up to concentration of 0.0001 mM.

INTRODUCTION

Polymeric micelles have received much attention in drug delivery, partly because of their unique characteristics such as easy control of particle size, good thermodynamic stability, ability to solubilize hydrophobic molecules, sustained drug release and prevention of rapid clearance by reticuloendothelial system (RES). In particular, the most promising application of polymeric micelles is their use as carriers for anti-cancer drugs. [1] Although numerous studies have shown large enhancement of antitumor activity of anti-cancer drugs (e.g., doxorubicin) by incorporation into polymeric micelles, the major problem of chemotherapeutic drugs is nonspecific toxicity against normal cells as well as tumor cells. Recently, active targeting has been attempted by many investigators to gain a high selectivity to a specific organ as well as to enhance the internalization of drug-loaded delivery systems into the targeted cancer cells. Different cancer cell-specific ligands such as sugars, biotin, vitamin H, vitamin B₁₂ and folic acid have been introduced into the drug carriers to improve delivery and retention of anti-cancer therapeutics with the tumor tissue. [1, 2]

Amphiphilic star-like-macromolecules (ASMs) are polymeric micelles in which the hydrophobic and hydrophilic polymer segments are covalently connected, and thus one molecule behaves as a single micelle entity. [3] Therefore, the micelle structure is static rather than dynamic and maintained at all concentrations and a variety of solvents. In this study, folic acid was introduced into the amphiphilic star-like macromolecules (ASMs) to improve their cancer targeting activity and internalization into cancer cells. Furthermore, in these preliminary studies, the ability of amphiphilic star-like macromolecules (ASMs) and folic acid-conjugated ASMs (FA-ASMs) to physically encapsulate doxorubicin (DOX) were examined as well as their cytotoxicity.

EXPERIMENTAL METHODS

Synthesis of folic acid-conjugated amphiphilic star-like macromolecules (FA-ASMs)

Based on our newly synthesized amphiphilic star-like macromolecules (ASMs) [4], folate moieties were added onto the poly(ethylene glycol) (PEG) shell. The detailed procedures are as follows and outlined in Fig. 1.

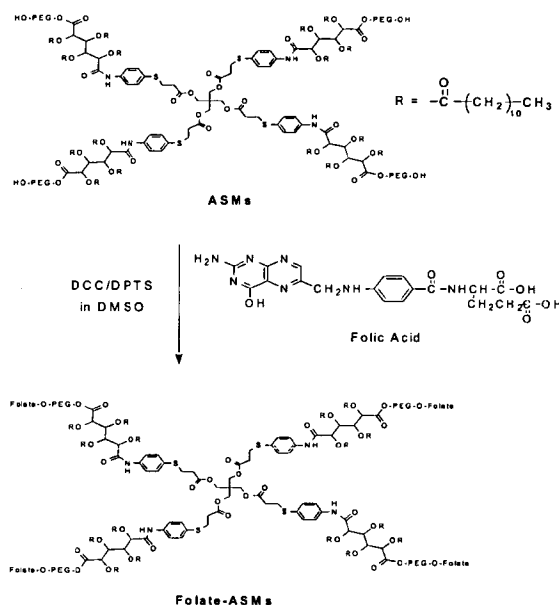


Fig. 1. Synthetic scheme of folic acid conjugated amphiphilic star-like macromolecules (FA-ASMs)

The ASMs with hydroxyl groups at the end of PEG (ASMs-OH) (1.0 g, 40 μ mol) and folic acid (0.20 g, 0.45 mmol) DPTS (0.16 g, 0.50 mmol) were added into 10 ml of DMSO at the room temperature. After 10 min flashing with argon, 1.0 ml of DCC solution (1.0 M in methylene chloride) was added dropwise over 15 min. After 24 h, the DCC side-product (dicyclohexylurea) was removed by suction filtration. After dilution with 10 ml of water, the resulting polymer solution was dialyzed against 4 L distilled water for 24 h by a Spectra/Por Cellulose Ester Membrane with a MW cutoff of 5 kDa, changing the water every 8 h. [5] A yellow powder was obtained after lyophilization. (0.40 g, 40% yield).

Preparation of DOX-loaded polymeric micelles

DOX-loaded ASMs and folic acid-conjugated ASMs were prepared by a dialysis method. Briefly, 2 mg of doxorubicin hydrochloride was dissolved in

0.2 ml of dimethylformamide (DMF) and added with 2 mol equivalents of triethylamine. 10 mg of polymer (ASM or FA-ASM) was dissolved in DMF (3.8 ml) and added to DOX solution. The resulting mixture was placed in dialysis bag (Spectrapor 2, Laguna Hills, CA) and dialyzed against 1 L of water for 24 h. The amount of entrapped DOX was determined by measuring the UV absorbance at 485 nm (DU 520, Beckman, Fullerton, CA). [1]

Cytotoxicity studies

Sensitive ovarian carcinoma cell line (A2780) was kindly provided by Dr. Minko (Department of Pharmaceutics, Rutgers University, Piscataway, NJ). The cells were cultured in RMPI 1640 media supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin solution for a minimum of two passages prior to the experimental use. They were subsequently harvested by trypsinization, resuspended in fresh media, seeded in 96-well plates at 5000 cells/well and cultured over 2 d to allow reattachment. The media was then replaced with fresh media containing various ASMs (0.0001-0.5 mM) and FA-ASMs (0.0001-0.03125 mM) concentrations. Following 24 and 48 h incubation at 37 °C and 5% CO₂, the cell survival was measured using standard MTT test. [2]

RESULTS AND DISCUSSION

Amphiphilic star-like macromolecules (ASMs) were modified by conjugation of folic acid onto its poly(ethylene)(PEG) shell. DCC/DPTS was applied as the dehydration agent. Due to the poor solubility of folic acid, the reaction was performed in DMSO. Excess of folic acid (3:1 molar) was added for the completion of the conjugation for the all four branches of ASMs. The dialysis method removed the excess folic acid. The NMR spectra confirmed the conjugation of folic acid to ASMs.

Doxorubicin (DOX) was successfully entrapped by physical manner into the hydrophobic inner core of ASMs and folic acid-conjugated ASMs (FA-ASMs) using a dialysis bag technique. The DOX encapsulation efficiency into ASMs and FA-ASMs were 15.0 % and 15.2 %, respectively.

The cytotoxicity of ASMs and folic acid-conjugated ASMs was evaluated *in vitro* using MTT assay and the results expressed as percentage of cell viability (%). The cytotoxicity studies showed that cell viability decreases proportionally to ASMs and FA-ASMs concentration (Fig. 2). Cell viability reached a value of approximately 60% and 50% after incubation of 24 and 48 h, respectively, for the highest ASMs concentration tested (0.5 mM). Folic acid-conjugated ASMs (FA-ASMs) began to exhibit cytotoxicity at a concentration of 0.01562 mM (Fig. 2b). After 24 and 48 h incubation with highest FA-ASMs concentration (0.03125 mM) the cell viability was 55% and 25%, respectively.

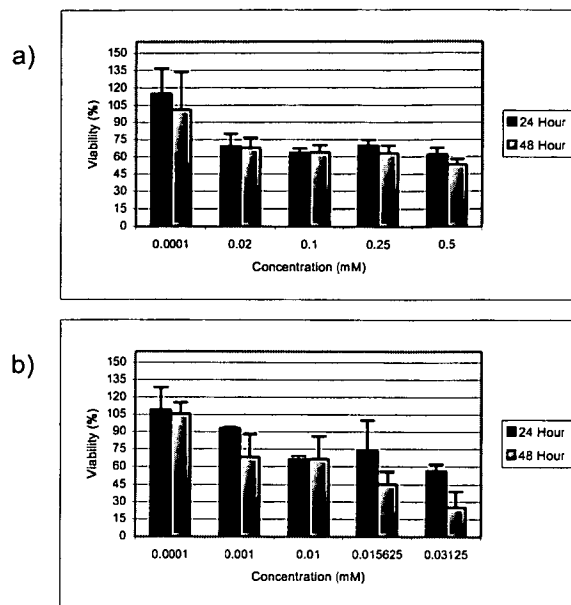


Fig.2. Viability of A2780 cells as a function of a) ASMs b) FA-ASMs polymer concentration (n=3).

CONCLUSION

We described an efficient synthesis of folic acid-conjugated ASMs (FA-ASMs). We also demonstrated that doxorubicin can be physically incorporated into the hydrophobic inner core of both ASMs and folic acid-conjugated ASMs. The cytotoxicity studies revealed that two-days cell incubation with ASMs and FA-ASMs polymer solutions didn't show significant cytotoxicity ($p < 0.05$) up to concentration of 0.0001 mM. The study of optimal loading conditions, release of DOX from the carrier and most importantly investigation of specific interaction of ASMs and folic acid-conjugated ASMs with sensitive (A2780) as well as resistant ovarian carcinoma cells (A2780 (AD)) cells will be undertaken and discussed in future publications.

REFERENCES

1. G. Kwon, T. Okano, Polymeric micelles as new drug carriers, *Adv. Drug Del. Rev.* 21 (1996) 107-116.
2. D. Dube, M. Francis, J. Leroux, F. Winnik, Preparation and tumor cell uptake of poly(N-isopropylacrylamide) folate conjugates, *Bioconjugate Chem.* 13 (2002) 685-692.
3. H. Liu, A. Jiang, J. Guo, K. E. Uhrich, Unimolecular micelles: synthesis and characterization of amphiphilic polymer systems, *J. Polymer Sci.: Part A: Polym. Chem.* 37 (1999) 703.
4. L. Tian, K. E. Uhrich, Design and synthesis of aliphatic-based amphiphilic star-like macromolecules for drug delivery (in preparation)
5. E. S. Lee, K. Na, Y. H. Bae, Polymeric micelle for tumor pH and folate-mediated targeting, *J. Control. Rel.* 91 (2203) 103-113.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

Claims

What is claimed is:

1. A method to target an anti-cancer compound to a cancer cell comprising, contacting said cell with a folic acid-conjugated amphiphilic star-like macromolecule encapsulating said anti-cancer compound.
2. A method to treat cancer comprising administering to a mammal a therapeutically effective amount of a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound.
3. A folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound.
4. The method of any one of claims 1 to 3, wherein the anti-cancer compound is doxorubicin.
5. The method of any one of claims 1 to 3, wherein the anti-cancer compound is 6-azauridine, 6-diazo-5-oxo-L-norleucine, 6-mercaptopurine, aclacinomycin(s), ancitabine, anthramycin, azacitadine, azaserine, bleomycin(s), capecitabine, carubicin, carzinophillin A, chlorozotocin, chromomycin(s), cladribine, cytarabine, daunorubicin, denopterin, docetaxel, doxifluridine, doxorubicin, edatrexate, eflornithine, elliptinium, enocitabine, epirubicin, etoposide, floxuridine, fludarabine, gemcitabine, idarubicin, mannomustine, melphalan, menogaril, methotrexate, mitobronitol, mitolactol, mitomycin C, mitoxantrone, mopidamol, mycophenolic acid, nogalamycin, olivomycin(s), paclitaxel, pentostatin, peplomycin, pirarubicin, piritrexim, plicamycin, podophyllinic acid 2-ethylhydrazine, prednimustine, procarbazine, pteropterin, puromycin, ranimustine, streptonigrin, streptozocin, teniposide, thiamiprine, thioguanine, Tomudex® (N-[[5-[[[(1,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]methylamino]-2-thienyl]carbonyl]-

L-glutamic acid), toptecan, trimetrexate, tubercidin, ubenimex, vinblastine, vindesine, vinorelbine, or zorubicin.

6. A pharmaceutical composition comprising a folic acid-conjugated amphiphilic star-like macromolecule encapsulating an anti-cancer compound, and a pharmaceutically acceptable diluent or carrier.
7. The pharmaceutical composition claim 6, wherein the anti-cancer compound is doxorubicin.
8. The pharmaceutical composition claim 6, wherein the anti-cancer compound is 6-azauridine, 6-diazo-5-oxo-L-norleucine, 6-mercaptopurine, aclacinomycin(s), ancitabine, anthramycin, azacitadine, azaserine, bleomycin(s), capecitabine, carubicin, carzinophillin A, chlorozotocin, chromomycin(s), cladribine, cytarabine, daunorubicin, denopterin, docetaxel, doxifluridine, doxorubicin, edatrexate, eflornithine, elliptinium, enocitabine, epirubicin, etoposide, floxuridine, fludarabine, gemcitabine, idarubicin, mannomustine, melphalan, menogaril, methotrexate, mitobronitol, mitolactol, mitomycin C, mitoxantrone, mopidamol, mycophenolic acid, nogalamycin, olivomycin(s), paclitaxel, pentostatin, peplomycin, pirarubicin, piritrexim, plicamycin, podophyllinic acid 2-ethylhydrazine, prednimustine, procarbazine, pteropterin, puromycin, ranimustine, streptonigrin, streptozocin, teniposide, thiamiprine, thioguanine, Tomudex® (N-[[5-[[[(1,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]methylamino]-2-thienyl]carbonyl]-L-glutamic acid), toptecan, trimetrexate, tubercidin, ubenimex, vinblastine, vindesine, vinorelbine, or zorubicin.